EFFECT OF PHYTOCHEMICAL CONCENTRATIONS ON BIOLOGICAL ACTIVITIES OF CRANBERRY EXTRACTS

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Plants of cranberry (*Vaccinium macrocarpon*) furnish edible fruits and derivates that have been used for the prevention and treatment of urinary tract infections. In the present work we compare two commercial extracts that contain proanthocyanins (PACs) at 4% and 20% for antimicrobial, antiproliferative, antiradical and protective properties against oxidative stress on cell lines. Both extracts showed antimicrobial activity (MIC values range 3-100 µg/ml). Extract at 20% PACs showed higher antiproliferative activity against HepG2 and MCF7 cells, but not against C2C12 cells. Both extracts showed a dose-dependent free-radical scavenging capacity, and a protective effect on the cell damage was also revealed by reduction of intracellular active oxygen species release. Cranberry extracts confirmed antioxidative proprieties and efficacy in reduction of cell viability that resulted stronger against tumor cells. The pretreatment with cranberry extracts, furthermore, reveal an increase of cell resistance against oxidative stress, suggesting a potential role as a dietary supplement in preventing free-radical damage. The proanthocyanidin content is critical to determine the extract efficacy. In cellular experiments the extracts resulted clearly differentiated in their activity, and the activity was strongly influenced by PACs content. Only in DPPH test the free radical scavenging activity seemed to be directly related to proanthocyanidins content.

The North American cranberry (*Vaccinium macrocarpon* Ait. Ericaceae) is a plant of growing public interest as a functional food because of its potential health benefits linked to phytochemicals in the fruit.

Cranberry juice has long been consumed for the prevention of urinary tract infections, and research linked this property to the ability of cranberry proanthocyanidins to inhibit adhesion of *Escherichia coli* bacteria responsible for infections. Anthocyanins possess important health benefits including antitumor, antiulcer, antioxidant, and anti-inflammatory activities (1). *V. macrocarpon* berries contain ascorbic acid (13.7 to 28.5 mg/100 g), phenolic compounds (192.3 to 676.4 mg/100 g), titratable acids (2.2% to 2.3%), and sugars (3.66% to 4.90%) (2-3). Composition, especially regarding phenolic fractions, is related to different activity and can vary significantly due to cultivars, rather than seasonal and physiological factors (4-6).

Key words: Vaccinium macrocarpon, cranberry, antiproliferative, antiradical, antioxidant

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In the present work we compared the biological activities, as antiradical, antiproliferative and protective effects against induced oxidative stress, of two commercial cranberry dry extracts characterized by different proanthocyanidins content (4% and 20%, respectively). The scope was to discriminate tested extracts on the basis of biological activities as direct effect of active metabolite concentrations.

To evaluate antioxidant activity, various *in vitro* tests are available, useful to determine the total antioxidant capability, but which cannot be considered predictive of *in vivo* efficacy. In the present study the antiradical activities were investigated in DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS [2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)] tests and in complex cellular systems, such as release of prostaglandins or reactive oxygen species.

Antiproliferative activities were determined and compared on tumor cell lines (HepG2 and MCF-7) and normal cell (C2C12). Protective efficacy against oxidative stress was determined as reduction of prostaglandin release and reactive oxygen species induced by hydrogen peroxide, from cells previously treated with extracts.

MATERIALS AND METHODS

Samples

Two samples of dry extracts of cranberries (*Vaccinium macrocarpon*) with different proanthocyanin content were obtained from the market. Both are sold as dry extracts standardized for the content in proanthocyanins and used as such or as an ingredient for formulations. The proanthocyanin (PAC) content resulted 4.01 ± 0.07 % (extract named C4%) and 20.04 ± 0.08 % (extract named C20%), respectively. The relative amounts of active phytochemicals are attested by specific analysis certificates and were determined by HPLC.

Antiradical activity

DPPH test (7). Scavenging free radical activity was tested in a methanol solution of (2,2-diphenyl-1-picrylhydrazyl (DPPH). The degree of decoloration of the solution indicates the scavenging efficiency of added extract samples. A methanol solution of extracts, 100 µl was added to 0.9 ml of pure methanol and 4 ml of DPPH solution (final concentration of DPPH: 2.0 x 10^{-4} M). Thirty minutes later, the absorbance was measured at 517 nm. A blank solution was prepared with 100 ml of methanol. The scavenging activity on DPPH radical was expressed as IC_{50}, that is the concentration of extract that allows a 50% decrease of the absorbance. Experiments were performed in triplicate.

$ABTS^{\bullet+}$ Assay (8). ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt, was dissolved in water to 0.7 mM concentration. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM (final concentration) potassium persulfate ($K_2S_2O_8$) and allowing the mixture to stand in the dark, at room temperature, for 12-16 h. The radical is stable in this form for more than two days, when stored in the dark at room temperature. For the study of antiradical activity of extracts, the $ABTS^{\bullet+}$ solution was diluted in ethanol up to absorbance of 0.70±0.02 at 734 nm. 100 µl of extracts solution (1 mg/ml) were added to $ABTS^{\bullet+}$ solution to a total volume of 1 ml and allowed to react for 5 min. Control (without sample) was used as blank. Trolox were used, as reference antioxidant substance (ranging concentration 0-15 µM). Appropriate solvent blanks were run in each assay. All determinations were carried out three times in triplicate. Results were expressed as millimoles of trolox equivalents.

Antimicrobial activity

The assessment of antimicrobial activity was performed on Gram-positive *Enterococcus faecalis, Staphylococcus* aureus, Gram-negative *Escherichia coli, Pseudomonas constantini, Proteus mirabilis* and yeast *Candida albicans* (9).

Bacteria were grown in nutrient agar broth (17g/L) or Mueller-Hinton agar (38g/L), for 24 h at 37°C. Yeast was grown in Sabouroud agar (65g/L). Medium were from (MHA, Oxoid). Plates were incubated overnight at 37°C and 33°C respectively for bacteria and yeasts.

Antimicrobial susceptibility of microorganisms was evaluated by the disk diffusion test. Stock culture of test bacteria and yeast were grown in medium at 37°C (33° for fungi) for 24 h. Final cell concentration was 0.5 McFarland Standard, determined by turbidometry (10). Plates were inoculated and a sterile paper disk of 6 mm diameter, filled with 100µg/mL solution of each extract, were gently placed on agar surface. The plates remain 1 h at room temperature and then incubated for 24 h. The inhibition zone around each disc was measured in millimeters. The assay was carried out three times for each extract. A solution of ethanol 80% was used as control.

Microorganisms with mean inhibition diameter over 8 mm were considered sensitive and used to determine the minimum inhibitory concentrations (MIC) performed by the agar dilution method according to guidelines provided by the NCCLS (11). Stock solution of cells were diluted with a sterile physiologic saline solution (0.9%

w/v sodium chloride) up to 0.5 McFarland standards, corresponding to 10^6 colony forming units (CFU) mL^{-1}. A serial dilution of extract solution was carried out to give final concentrations ranging 3.125-100 µg/mL in culture mediums. Microplates were inoculated with 10 µL of the bacterial suspension and incubated. The minimum inhibitory concentration (MIC) value was determined as the lowest concentration that inhibited the visible growth of the tested microorganisms.

Cell culture

Human breast cancer (MCF-7), liver cancer (HepG2) and mouse myoblast cells (C2C12) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% streptomycin, in humidified atmosphere of 5% CO_2 at 37°C, until confluent.

The culture medium was changed twice every week, and the cells were subcultured at 1:4 ratio, once a week.

Biopsy from *Vastus lateralis* (VL) muscle was obtained from a male healthy non-trained patient (69 years of age) who underwent elective orthopedic surgery after informed consent. Biopsy (muscle pieces 0.1–0.2g in weight) was performed using the procedure of Engel (12), and immediately treated to obtain explants, as already described (13). The biopsy sample was collected in Ham's F-10 medium (GIBCO, Invitrogen, Carlsbad, California) supplemented with 50 µg/ml of gentamycin and stored at +4°C until processing, which was performed within 24 h of surgery. The first mononucleated cells migrated out of the explants within 7–13 days from the beginning of culture.

Cell viability

Cell viability was determined, as previously described (14), by the reduction of yellow 3-(4,5-dimethylthaizol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma Chemical Co.) into a purple formazane product by mitochondrial dehydrogenase of metabolically active cells. Briefly, cells were seeded at a density of 2.5×10^5 cells/well into a 96-well plate. After overnight growth, cells were treated with freshly prepared medium containing extracts at ranging concentration 50-100µg/ml for 48 h. At those times, 20 µL of MTT were added, and incubation prolonged for 4 h, then plates were centrifuged (900 g for 5 min) and supernatants were removed. The pellets were dissolved in 200 µl dimethylsulfoxide and the absorbance read at 540 nm on a scanning multi-well spectrophotometer (Cary50MPR, Varian). Each experimental condition was repeated in sixteen wells and the experiments and measurements were performed in triplicate. The median effective concentration (IC_{50}) was calculated on mean values and corresponds to extract concentration that allows inhibition of cell proliferation by 50%.

Protective effect against oxidative stress

In order to detect the efficacy of extracts in preventing damage induced by oxidative stress, cells incubated in presence of subtoxic doses of extracts were stimulated by H_2O_2, and measured cellular effects in terms of prostaglandins release.

1 mg of extracts were dissolved in 1 ml DMSO (stock solution) and serially diluted in sterile medium for cell growth to achieve concentration range 50-100 µg/ml. The final concentration of DMSO was <1%.

Cells were plated in multiwell culture plates at 1.5×10^5 cells per well. After 48 h cells were stimulated with 2 µL of hydrogen peroxide solution (0.5 mM) for 15 min. After treatment plates were centrifuged and supernatant used for isoprostane release quantification. In preliminary experiments cell viability was determined by trypan blue test in order to exclude, at working concentration, toxic effect due to H_2O_2. Oxidative stress effects were detected as increased in PGE2 release compared to the untreated control group. Protective effects were determined as percentage reduction of PGE_2 release induced by H_2O_2, in groups incubated in presence/absence of extracts.

Prostaglandin E_2 radioimmunoassay

Prostaglandin RIA was performed according to Ciabattoni et al. (15), 100 µL of prostaglandin E_2 standard or sample was incubated overnight at 5°C with the respective ^{3}H-prostaglandin (3000 cpm/tube; NEN) and antibody (final dilution: 1:80000; kindly furnished by Prof. Ciabattoni), in a volume of 1.5 ml of 0.025 M phosphate buffer. Free and antibody-bound prostaglandins were separated by the addition of 100 µl 5% bovine serum albumin and 100 µL 3% charcoal suspension, followed by centrifuging for 10 min at 4000 x g at 5°C and decanting off supernatants into scintillation fluid (Instangel Plus) for β emission counting. Sensitivity of the assay was 2pg/ml; ED50 was 20 pg/ml. The PGE_2 production was expressed as picograms per ml.

Measurement of intracellular reactive oxygen species (ROS)

Cellular ROS were quantified by the 2,7-dichlorofluorescin diacetate (DCFH-DA) assay using a microplate reader. DCFH-DA is a useful indicator of reactive oxygen species (ROS), in order to determine the antioxidant properties of foods, extracts and molecules. DCFH-DA is a non-fluorescent ester dye that penetrates the cells and is hydrolyzed by intracellular esterases to the DCFH, which can be rapidly oxidized by ROS (as H_2O_2) to the highly fluorescent 2,7-dichlorofluorescein (DCF) in the

presence of ROS. For the assay, all cells lines were plated in black 96-well at a rate of 2 × 10^5 cells per well. Twenty-four hours later, the medium was changed and medium with extracts at ranging concentrations 50-100µg/mL per well was added and incubated at 37°for 48 h.

Thereafter, the cells were washed 3 times with DPBS and 100 µL of 1X DCFH-DA media solution were added and the plates were incubated at 37ºC for 40 minutes. After 2 washings with DPBS cells were treated with 100 µL of oxidant solution (H_2O_2 0.5 mM, in DMEM) for immediate fluorescence measurement. The fluorescence intensity is proportional to the ROS levels within the cell cytosol. Plates were read for kinetic analysis in increments from 1 to 5 minutes every 30 seconds. Fluorescence was measured by Microplate Fluorometer SPECTRAmax Gemini XS (Molecular Devices, Sunnyvale, CA, USA.) using excitation and emission wavelengths of 480 nm and 530 nm, respectively, and analyzed by SOFTmax Pro software.

Statistical analysis

Experimental values are reported as means and standard deviation (SD). Analysis of variance and significances were performed by One-way ANOVA with Newman-Keuls Multiple Comparison post test, using GraphPad Prism version 5.00, (GraphPad Software, San Diego, California, USA). $P < 0.001$ was considered statistically significant.

RESULTS

Antiradical activity

In the $ABTS^{\bullet+}$ assay, the efficacy of cranberry extracts solutions 1mg/ml, expressed as millimoles of trolox equivalents, resulted 69.58 and 218.70, respectively, for the extract containing 4% and 20% of PACs. At the same concentration a solution of butylated hydroxytoluene (BHT), used as reference antioxidant, give a value of 97.26 mmol of trolox equivalents.

The EC_{50} calculated in DPPH test results 55±7, 9 ±1 and 12 µg±2 respectively for C4%, C20% and for trolox, used as reference antioxidant.

Both extracts exhibit high antiradical capacity, with values close to or better than reference antioxidant. Much more efficacy was revealed by C20% extract, which always resulted more efficient than reference drugs and from 3 to 5 times more active than other extracts.

Antimicrobial activity

The strain susceptibilty in the disk diffusion test revealed that the extract standardized at 4% partially inhibited only *Proteus mirabilis*, *Candida albicans* and *Staphylococcus aureus* growth, while all strains were susceptible to C20% extract.

Data of MIC determination are referred only to C20% extract, while C4% extract failed inhibitory activity also at the highest concentrations tested (100µg/ml). Experiments were developed in triplicate.

Only *Enterococcus faecalis* result not sensible

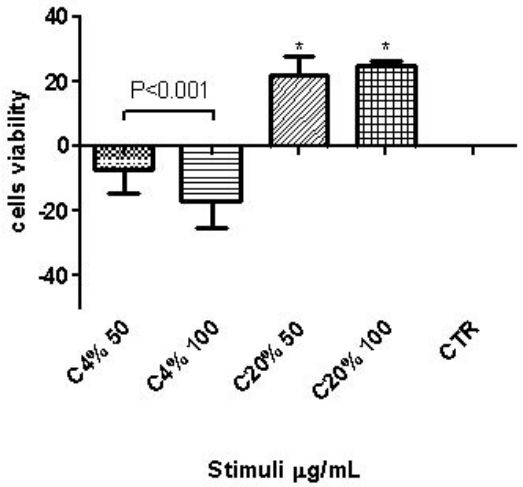

Fig. 1. *C2C12 Cell viability, % variation vs control – 48 h. C4%, C20%: Cranberry 4% and 20% PAC's extracts Numbers indicate stimuli concentrations, expressed as µg/ml ANOVA P<0.0001, *P<0.001 vs control*

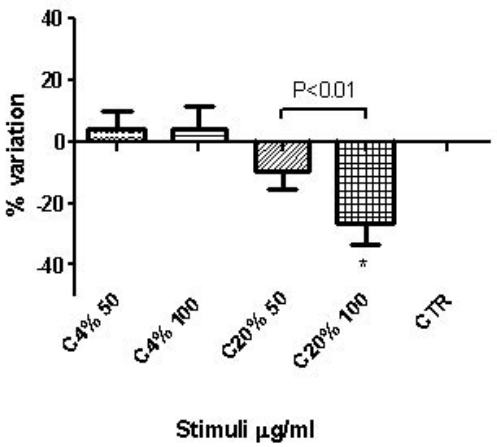

Fig. 2. *HepG2 Cell viability, %variation vs control – 48 h. C4%, C20%: Cranberry 4% and 20% PAC's extracts Numbers indicate stimuli concentrations, expressed as µg/ml ANOVA P<0.0001, *P<0.001 vs control*

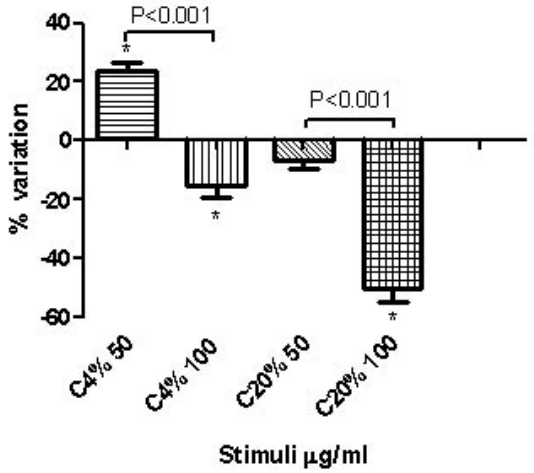

Fig. 3. *MCF-7 Cell viability, % variation vs control – 48 h. C4%, C20%: Cranberry 4% and 20% PAC's extracts Numbers indicate stimuli concentrations, expressed as µg/ml ANOVA P<0.0001, *P<0.001 vs control*

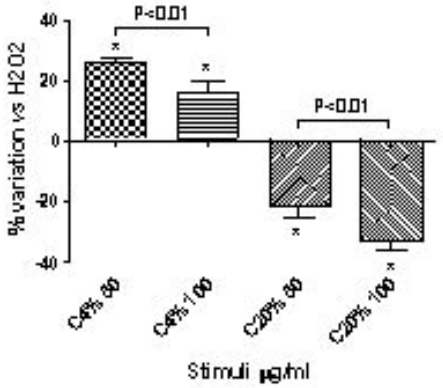

Fig. 4. *MCF-7, PGE_2 release, % variation vs untreated – 48 h. C4%, C20%: Cranberry 4% and 20% PAC's extracts Histogram reports percentage variation of PGE_2 release vs control, in groups stimulated by H_2O_2. Numbers indicate stimuli concentrations, expressed as µg/ml ANOVA P<0.0001, *P<0.001 vs control*

to C20% extract, while all the others were inhibited, even if the value revealed a weak antimicrobial activity.

The MIC values resulted 100 µg/ml for *E. coli* and *S. aureus*, 25 µg/ml for *P. mirabilis* and *C .albicans*. The most subsceptible strain was *P. constantini* that resulted inhibited up to 12.5 µg/ml.

The antimicrobial activity seemed to be strictly related to proanthocyanidins content that influence activity spectrum and growth inhibition efficacy of

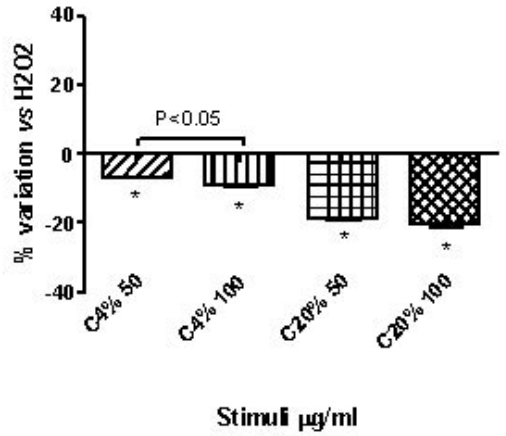

Fig. 5. *HepG2, PGE_2 release, % variation vs untreated – 48 h. C4%, C20%: Cranberry 4% and 20% PAC's extracts Histogram reports percentage variation of PGE_2 release vs control, in groups stimulated by H_2O_2. Numbers indicate stimuli concentrations, expressed as µg/ml ANOVA P<0.0001, *P<0.001 vs control*

the extracts. In fact, even if both extract showed strain susceptibility, higher PAC content afforded a broad activity spectrum and lower MIC values.

Cell viability

C2C12

Experiment of viability on cells line C2C12 revealed different activity of two cranberry extracts. After 48 h of incubation, the less concentrated extract, compared to controls, induced a slight reduction of cell viability, even if with no clear dose-dependent effect while, in opposition, C20% extract seemed to always stimulate cell proliferation (Fig. 1).

HepG2

HepG2 cells were cultured and stimulated with cranberry extracts, and after 48 h cell viability was determined by MTT assay. At 48 h C20% extract revealed a dose-dependent antiproliferative activity, while C4% did not seem to significantly affect cell viability. The effect was evident also comparing the theoretical calculated IC_{50} which was 159.4 for C20% extract while the other extract was not effective (Fig. 2).

MCF-7

After 48 h of incubation, both extracts exhibited

Table I. *ROS release, % variation vs untreated – 48 h.*

	HepG2	C2C12	Biopsy
C4% H2O2	-49.06±8.58*	-26.52±11.34	51.02±19.32
C20% H2O2	-83.44±15.31*	-64.84±20.38	4.06±9.07

C4%, C20%: Cranberry 4% and 20% PAC's extracts
Mean percentage variation of ROS release vs control, in groups stimulated by H_2O_2.
Extracts were tested at 100 µg/ml
*ANOVA P<0.0001, *P<0.001 vs control*

antiproliferative activity, but only at higher doses. C20% extract resulted more effective, reducing the cell viability up to 50% at 100 µg/ml concentration (Fig. 3).

The viability experiments revealed on significant difference all cells lines between the two tested extracts that can be related to proanthocyanidin content. In experiments on tumor cell line (HepG2 and MCF-7), C20% extract showed more efficacy in reduction of cell viability, with effects that were not directly related to the amount of active principles. On C2C12 mouse muscle cells the effects of two extracts were completely different, in fact the cell viability resulted slightly reduced by C4% at higher concentration, while it was not affected, or at least as in a minimal percentage, by extracts with higher proanthocynidin content.

PGE_2 release

Based on strict relation between tumor process and oxidative metabolism, the effect of pre-incubation of cells in presence of extracts in culture medium, were assayed as variation of release of oxidative marker, such as isoprostane. Experiments were not performed on non-tumor C2C12 cells that resulted, as described above, poorly influenced by extracts. Cells incubated in the presence of different amounts of extracts were stimulated with hydrogen peroxide in order to induce an oxidative stress that can be revealed in culture medium as increase of prostaglandin PGE_2 released compared to unstimulated control groups. Quantification of PGE_2 were developed via radioimmunologic assay.

MCF7

After oxidative stimulation induced by H_2O_2, all stimulated groups increased isoprostane release. Compared to cells incubated in absence of extracts, an evident effect due to pretreatment of cranberry was evident at 48 h, when the effects of two extracts was clearly different.

Lower doses of C4% extract increased the PGE_2 release that at 100µg/ml was reduced by over 40%. The C20% extract, at highest doses, reduced the release of prostaglandins induced by the oxidative stimulus. This can be considered as a protective efficacy of pretreatment with extracts to prevent oxidative stress induced by external stimuli (Fig. 4).

Treatment with C4% extract failed to afford a protective effect. At all doses an increase in PGE2 release was recorded, while the C20% extract resulted as being able, at higher doses, to reduce the release of prostaglandins induced by the oxidative stimulus. This result can be considered as a protective efficacy of pretreatment with extract to improve cell resistance to oxidative stress induced by external stimuli.

HepG2

Groups of HepG2 cells incubated in presence of extracts showed a reduction of the PGE2 release compared to the control groups considered as reference for basal release (data not shown). The stimulation with H_2O_2 induced a significative increase, at least 50%, of isoprostane release as resulted in the control group not pretreated with extracts. The tested extract afforded different efficacy in protection of cells against the oxidative stress. Both extracts afforded a significative reduction in PGE2 release. C4% extract also gave a significative dose-reduction response, but resulted less effective than C20% extract, which induced a reduction, two

times stronger than prostaglandin release (Fig. 5).

ROS Release

In order to demonstrate whether the protective activity was due to a single mechanism rather than to a complex system, a similar experimental model was developed to highlight the protective effects against hydrogen peroxide stimulation revealed as reduction of intracellular reactive oxygen species production. The results are reported in Table I.

HepG2

Stimulation of HepG2 cells with hydrogen peroxide (0.5 mM) induced a marked increase of ROS production (over 50%, data not shown). A preliminary incubation with 100 µg/ml extracts afforded an evident protective effect that was revealed by a strong reduction of C4% extract and fully inhibited by C20% extract (reduction over 100%, compared to group stimulated with H_2O_2, in absence of extract).

C2C12

In C2C12 cells, only C20% treatment gave antioxidant protection. In presence of C4% no differences were revealed in respect to untreated groups (data not shown), while C20% significantly reduced the ROS release induced by hydrogen peroxide in a dose-dependent manner.

Human myoblasts

The protective effects of C20% extract, that resulted of higher interest for protective effect against oxidative stress, were also tested in culture of human myocytes. Cells stimulated by hydrogen peroxide responded with an evident increase of ROS release. The cells cultured in medium enriched with extracts afforded a stronger resistance to cells against oxidative stress, as revealed by marked (over 90%) reduction of ROS release. This experiment confirmed the potentiality of cranberry as a protective agent against exogenous oxidant.

DISCUSSION

In the present work biological activity of two cranberry extracts, characterized by 4 and 20% of PAC content, were compared. In all experiments performed, the extracts gave a different activity, both qualitatively and quantitatively. In antimicrobial activity experiments, the highest PAC concentration seemed to give a broad activity spectrum and resulted more efficient in inhibition of tested strains. Weak MIC values confirmed literature data that report cranberry has no elective phytocomplex for antimicrobial therapy.

Huge consumption of cranberry is related to well-known antioxidant activity of extracts and phytochemicals, mainly related to anthocyanins (1). In literature it is highlighted that the antioxidant activity of berries varies considerably, related to factors like genotype and cultivars, maturation, season (17, 4-5) and is also correlated to anthocyanins or phenolics content (6, 18).

In the antiradical ABTS and DPPH tests, both extracts confirmed the antioxidant activities with values that were close or higher than reference drug. PAC concentrations in the extracts exerted an evident direct influence on activities that resulted more than three times higher in the most concentrated extract. Antiproliferative and antioxidant activity of cranberry extract is also confirmed in literature data. Isolated phytochemicals from cranberry fruits showed antiproliferative activity, but the relationship between phytocomplex and active principle activity requires further investigations. The antiproliferative activity is attributable to the complex mixtures of phytochemicals present in whole extracts rather than in specific chemicals (19-21).

In this present work, cell viability tests were performed on three cell lines, C2C12 muscle cells, HepG2 hepatocellular carcinoma and MCF-7, pleural effusion adenocarcinoma. In all tests, the activity of extracts resulted clearly differentiated. In C2C12 cell line, an increase of cell proliferation was observed in presence of increasing doses of higher concentration extract, while the C4% extract induced a reduction in cell viability. On the other hand, against tumour cell lines, the antiproliferative activity was stronger for C20% extract. In HepG2 cell, the low concentration of extract did not exhibit a reduction in cell viability that resulted clearly evident for the more concentrated extract, with a dose-dependent response. In MCF-7 cells, the antiproliferative activity was revealed only at higher extract doses, and was clearly more evident for C20% cranberry. These results confirm

the antiproliferative role of the extracts on tumour cell lines, as demonstrated with other substances that have known antioxidant properties (22-24) and are perhaps in accordance with previous literature data where cranberry extracts and their phytochemicals are considered more effective in reduced cell viability of tumor rather than of no tumor cell lines (15).

To investigate the relationship to oxidative stress in tumour cells, the effect of extracts pre-treatment was studied, in reduction of oxidative damage induced by endogenous stimulus. Oxidative stress plays a key role in the defense mechanisms of cells, but it is also involved in the pathogenesis of many diseases related to cell degeneration, chronic inflammation, cell aging etc. Prostaglandins are a class of compounds derived from arachidonic acid consequent to oxidative reaction mediated by cycloxygenase (25). The prostaglandin formation can be induced by different endogenous stimuli, such as oxidative chemicals, and quantification of prostaglandins release can be used as a reliable index of cellular oxidative damage (15). In the present study, the efficacy of cranberry treatment is revealed as percentage reduction of prostaglandin release induced by hydrogen peroxide. Extract activity results clearly differentiated. In HepG2 cells, both extracts were able to reduce PGE2 release, with stronger inhibition always related to C20% extract. The MCF-7 cell line, where C4% extract did not show inhibition, resulted as being less sensitive, while the more concentrated extract was active only at higher doses.

Extract activity wasclearly differentiated also in a similar antioxidative test, where the activity was revealed as reduction of intracellular release of reactive oxygen species induced by hydrogen peroxide. In C2C12 cells, only C20% extract reduced the ROS release, while in HepG2 cell line both extracts were active, but the more concentrated extract showed a significative stronger activity. Both tests confirmed the ability of pre-incubation of cells with cranberry extracts as a protective treatment that gives cells stronger resistance against oxidative stress, and clearly demonstrated that efficacy of extracts is related to PACs content.

It is known that the myoblasts derived from the elderly have higher levels of oxidative stress due to reduced antioxidant activity (13). For this reason, the effect of the extract was analyzed to verify antioxidant activity also in cells derived from an elderly man. The antioxidant activity of higher concentration extract resulted clearly evident also on human myoblasts isolated from a male (69 years old) and exposed to oxidative stimuli. Incubation of cells in presence of C20% extract give an evident protective effect that completely neutralized the increased production of ROS induced by hydrogen peroxide.

The marked antioxidant effect of these extracts (mainly 20% PACs) shown on all cells would be related to the antiproliferative role that they accomplish. This role in normal cells is not necessary and the antioxidant effect is only for protection by radicals stimulating the proliferation, but in tumour cells could be useful to activate mechanisms that inhibit the proliferation.

In conclusion, we compared the activities of two commercial cranberry extracts by antimicrobial and antiradical tests and on cellular systems, such as cell viability and antioxidant capability. Extracts confirmed the well-known antioxidant activity of cranberries both in chemical and biological systems. In all experiments the efficacy of the two extracts were clearly differentiated, in terms of activity/inactivity or in terms of stronger activities that always resulted related to the higher concentration in active phytochemicals, such as proanthocyanins.

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